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Preferential adhesion of surface groups of *Bacillus subtilis* on gibbsite at different ionic strengths and pHs revealed by ATR-FTIR spectroscopy

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ABSTRACT

Adhesion of bacteria onto minerals is a ubiquitous process that plays a central role in many biogeochemical, microbiology and environmental processes in soil and sediment. Although bacterial adhesion onto soil minerals such as phyllosilicates and Fe-oxides have been investigated extensively, little is known about the mechanisms for bacterial attachment onto Al-oxides. Here, we explored the adhesion of *Bacillus subtilis* onto gibbsite (γ-AlOOH) under various ionic strengths (1, 10, 50, and 100 mM NaCl) and pHs (pH 4, 7, and 9) by in-situ attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. The time evolution of the peak intensities of the attached bacteria suggested that the adhesion underwent an initial rapid reaction followed by a slow pseudo-first-order kinetic stage. Spectral comparison between the attached and free cells, together with the interaction energy calculated with the Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory and the micro-morphology of bacteria-gibbsite complexes, indicated that both electrostatic and chemical (bacterial groups such as phosphate and carboxyl covalently bind to gibbsite) interactions participated in the adhesion processes. Both solution ionic strength (IS) and pH impacted the spectra of attached bacteria, but the peak intensity of different bands changed differently with these two factors, showing a preferential adhesion of surface groups (phosphate, carboxyl, and amide groups) on gibbsite at different conditions. The diverse responses to IS and pH alteration of the forces (chemical bonds, electrostatic attractions, and the hydrophobic interactions) that essentially govern the adhesion might be responsible for the preferential adhesion. These results may help to better understand how bacteria adhere onto soil oxides at molecular scales.

1. Introduction

Almost 80–90% of the microorganisms in soil and waters are attached to solid surfaces, rather than being free cells in aqueous matrix [1]. This attachment plays a vital role in a broad variety of biogeochemical, microbiology and environmental processes, such as formation and weathering of minerals, biofilm formation, biodegradation of organic pollutants, and activity and transport of pathogen [2]. In addition, the bacteria-mineral interactions have been reported to affect the adsorption and speciation of both heavy metals (e.g. Cu) [3] and oxyanions (e.g. As and P) [4,5] on oxides, thus probably regulating their distribution and bioavailability in soils and sediments. Therefore, it is essential to understand to which extent and how bacteria adhere to solid surfaces.

Attachment of bacteria onto solid surfaces roughly includes an abiotic initial adhesion process and a subsequent biological process which ultimately leads to biofilm formation [2]. Over the past decades, extensive studies have been done on the initial adhesion of bacteria onto a variety of solid surfaces. In early studies, the most examined surfaces were inert and artificial solids such as glass, polystyrene, quartz, and corundum [2,6–8]. The bacterial adhesion on these surfaces has been considered as a physicochemical process which can be well described by theories of colloid chemistry, such as the classic Derjaguin, Landau, Verwey, and Overbeek (DLVO) or the extended-DLVO theory [9,10]. These theories are inclusive of the main forces that essentially dominate the adhesion, including electrostatic force, Lifshitz–van der Waals interactions and acid–base interactions [10,11].

However, the aforementioned inert solids differ greatly from soil particles in their properties and composition. In later studies, more attention was paid to bacterial adhesion onto clay-sized minerals, which are the major solid surfaces exposed to bacteria in soil. Using

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density-gradient centrifugation approaches, the extent of adhesion of bacteria on phyllosilicates and oxides was quantified [12,13]. A much larger number of cells adhere on Fe-oxides such as goethite than clay minerals such as kaolinite and montmorillonite, while primary minerals such as quartz have the least adhesion [12–14]. The attachment of bacteria on phyllosilicates can be generally predicted by the classic or extended DLVO theory, whereas bacterial adhesion on goethite cannot be fully explained by these theories [15]. Further ATR-FTIR spectroscopic studies revealed that the bacterial adhesion on goethite involves not only the extended DLVO interactions but also the covalent bonding between their surfaces [4,16–18]. Inner-sphere bonds have been found to form between bacterial phosphate and carboxyl groups and goethite hydroxyls. Similarly, bond formations were also seen during bacterial adhesion onto other Fe-oxides such as hematite [5,18–21].

Besides Fe-oxides, the most active inorganic solids exposed to bacteria in soil include Al-oxides such as gibbsite. Generally, aluminol groups (>Al−OH) of Al-oxides have a stronger reactivity towards orthophosphate than iron hydroxyls (>Fe−OH) [22]. Therefore, it is reasonably to infer that the surface groups on bacteria such as phosphate may also bind to the hydroxyls on Al-oxides, thereby resulting in a strong attachment between bacteria and Al-oxides. However, few studies have focused on the adhesion of bacteria on Al-oxides. Batch experiments showed that more nano-sized γ-Al_2O_3 particles adhered to the surface of *Escherichia coli* as compared to nano-sized α-Fe_2O_3 particles, and the attachment of bacteria with both particles generally decreased with increasing pH and decreasing ionic strength (IS) [23]. Recently, we found a nearly 100% adhesion of bacteria onto gibbsite at pH 5.0 in 1 mM NaCl solution and an inhibitory effect of bacteria on orthophosphate adsorption on gibbsite [24]. These experiments suggest a strong interfacial interaction between bacteria and gibbsite, however, the information regarding the adhesion mechanisms is quite lacking.

Hence, this study aims to explore the mechanisms of bacterial adhesion to Al-oxides under varying solution pH and IS by using in-situ ATR-FTIR spectroscopy. Gibbsite (γ-AlOOH), a predominant Al-oxides in variable charge soils from humid tropical or subtropical regions, and *Bacillus subtilis*, a common bacteria strain in soil, were used as representatives. Adhesion processes of *B. subtilis* onto gibbsite were monitored in real time, and the spectra of the attached cell and the free ones were compared to investigate the involvement of surface groups in the adhesion. We also compared the spectra collected at different pH and IS, to determine their influences on the adhesion. In addition, the adhesion kinetics under varying solution conditions were analyzed by plotting the peak intensity against the reaction time.

2. Materials and methods

2.1. Bacterium and gibbsite

B. subtilis was obtained from the China General Microbiological Culture Collection Center (CGMCC 1.88, Beijing, China). The bacteria were prepared following our previous study [4], and the details are provided in the Supplementary data.

Gibbsite was synthesized according to Rosenqvist et al. [25]. Briefly, 1.0 M $AlCl_3$ solution was titrated into 4.0 M NaOH solution under constant stirring until its pH reached about 4.6 to produce a suspension of amorphous aluminum hydroxide. The resulting slurry was aged at 40 °C for 2 h before electro-dialysis against ultrapure water at room temperature for 3 months. Finally, the minerals were dried at 60 °C and ground to pass through a 149-μm mesh sieve. Powder X-ray diffraction analysis (Fig. S1) and scanning electron microscopy (SEM) (Fig. 4) confirmed that the material was gibbsite with good crystallinity and purity.

2.2. In-situ ATR-FTIR spectroscopy

Adhesion of *B. subtilis* onto gibbsite was examined in situ by an ATR-FTIR spectroscopy with a multi-reflection horizontal trough plate using a ZnSe crystal as internal reflection element (IRE) (Nicolet 8700 spectrometer, Thermo Fisher Scientific, MA, USA) following a method similar to the one previously described [5]. Briefly, 0.4 ml of sufficiently dispersed gibbsite suspension (1 mg ml^{-1}) was deposited on a ZnSe crystal trough plate, resulting in a stable thin film (about 0.8 μm thick) evenly spread across the crystal surface. After equilibrating the gibbsite film with the background electrolyte solution (i.e. NaCl solutions pre-adjusted to the desired concentrations and pHs) for 1 h, a spectrum was collected as the blank spectrum. Afterwards, 0.2 ml of 5 mg ml^{-1} ($\sim 5 \times 10^8$ cells ml^{-1}, in the background electrolyte solution) bacteria solution was added to the reaction vessel after gently discarding the NaCl solution. Spectra were then recorded in a certain time interval until no significant spectral changes were observed. A set of adhesion experiments of bacteria on gibbsite films were conducted, at ionic strengths ranging from 1 to 100 mM (fixed pH = 7), or pHs ranging from 4 to 9 (fixed NaCl concentration = 1 mM). Spectra of aqueous bacteria and gibbsite on bare ZnSe surface were also collected under the same conditions. All these experiments were performed at 25 °C.

Each spectrum was scanned 200 times at a 4 cm^{-1} resolution over the range of 4000–600 cm^{-1}. The net spectra for adsorbed bacteria were obtained by subtracting the blank spectrum from the corresponding original sample spectra with a subtraction factor of 1.0. All manipulations with the spectra were performed using OMNIC 8.0 software package (Nicolet Analytical Instruments, Wisconsin, USA).

ATR-FTIR spectroscopy permits investigation of dipolar functional groups only in close proximity to the sample–IRE interface. The probing depth depends on the penetration depth of the infrared evanescent wave, the amplitude of which decays exponentially with distance from the IRE surface [26]. On the basis of the incidence angle (45°), the refractive index of ZnSe (2.4) and the bacterial cells (1.39), the penetration depth of the infrared evanescent wave at 1800 and 800 cm^{-1} is about 0.9 and 2.0 μm, respectively, as estimated following the previously reported method [26]. The thickness of the gibbsite deposit on the trough ZnSe plate was estimated at 0.75 μm, based on the amount of gibbsite deposit (0.4 mg), the density of gibbsite (2.44 g cm^{-3}) and the base area of the trough cell surface (2.1 cm^2). The *B. subtilis* cells used here are rod shaped with a size of about 2 μm × 0.5 μm. It has been evaluated by total internal reflection aqueous fluorescence microscopy that immobilized bacteria on inert surfaces like quartz are typically 20–90 nm far from the surface [27,28]. Therefore, the evanescent wave is expected to penetrate the entire thickness of the gibbsite film and probe at most only one layer of the bacterial cells which is close enough to it. For an infrared spectrum, the outmost bacterial surface groups, i.e. the groups reacted with the gibbsite or ZnSe surface, give a much more contribution than the internal or non-reacted groups. In this sense, the acquired bacterial spectra generally arise from the groups that really interact (physically, chemically or both) with the ZnSe or gibbsite surface.

2.3. DLVO calculations

DLVO theory (sphere–plate model) was applied to calculate the interaction energy between the bacteria and gibbsite under the same range of ionic strength or pH as used in the above ATR-FTIR experiments. Calculation details are given in the Supplementary data.

Hydrodynamic diameter of *B. subtilis*, one of the parameters needed for the calculation, was determined to be about 0.5 μm